CA3 ON HW Q90
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INTERIM REPORT - UPPER OTTAWA
STREET LANDFILL SITE STUDY,
REFERENCE PAPER 16 :
ANALYSIS OF AIR PARTICULATES
& LEACHATES ...

MUTATECH INC.

REPORT: THE ANALYSIS OF AIR PARTICULATES AND LEACHATES FROM THE UPPER OTTAWA STREET LANDFILL FOR MUTAGENICITY IN THE AMES TEST

Reference 16.

November 1982

P.O. BOX 198 KLEINBURG, ONTARIO, CANADA L0J 1C0

SUMMARY

Air particulate and leachate samples collected on or around the Upper Ottawa Street Landfill Site have been analysed for mutagenicity in the Ames assay.

In experiments with TA98 and TA1537, in no case did the mutagenicity of extracts from air particulates collected on the site exceed the mutagenicity of air particulates obtained at control locations around Hamilton. Although the highest value for mutagenicity using TA100 was obtained with an extract from particulates collected on the landfill, this result could not be repeated and may have been a false positive. Activity of other particulate extracts on TA100 was low.

Only one clearly positive result was found amongst the leachate samples analysed. A doubling of background reversion frequency was obtained using leachate from bore hole #4 and TA98.

INTRODUCTION

This report provides details of the analysis of leachate and air particulate samples collected on or around the Upper Ottawa Street Landfill Site for mutagenicity in the Ames assay.

MATERIALS & METHODS

Mutagenic activity was assayed using histidine auxotrophs of S.typhimurium. Strains TA98,TA100,TA1535,TA1537 and TA1538 were kindly provided by Dr. Bruce Ames, Berkeley, Calif. and were stored as frozen stocks at -70°C. The characteristics of these stocks are checked periodically (Histidine requirement,rfa character, R-factor,uvrB deletion: Appendix).

Bacterial cultures for air particulate studies were established by scraping a sterile wooden applicator stick across the surface of the frozen stock, innoculating either 5 or 20ml nutrient broth and shaking overnight at 37°C. Cultures for leachate studies were from a 1/100 dilution of an overnight culture shaken for 6hr at 37°C.

Air Particulate Samples

Air particulates were collected using "hi-vol" samplers, provided by the MoE, operating over a 24hr period from noon Aug. 11,1982 to noon Aug.12th. Filters were delivered to Mutatech Inc. in manilla envelopes and were stored in the dark at 4°C. Before extraction each filter was left at room temperature for 48hrs,then weighed. Particulate weights are shown in Table I. Filters were extracted according to the method of Katz et al 1978.

Each filter was folded and placed in a glass extraction thimble with a sintered glass disc at one end. This was then placed in a Soxhlet extractor and extracted for 17 hours with 200ml methanol. After extraction the thimble was allowed to cool to room temperature and rinsed with 25ml methanol; all washings were collected in the boiling flask and the contents of the flask filtered through a sintered glass funnel. Flask and funnel were then rinsed 3x with 7ml methanol and the entire extract reduced to about 2ml on a rotary evaporator in a 50ml pear-shaped flask. The water bath temperature was maintained at approx. 30°C. The remaining solvent was then transfered to a glass vial, the pear-shaped flask rinsed 3x and the final extract evaporated to dryness under a stream of nitrogen. The residue was stored in the dark in the refrigerator.

Ames Assay

Each extract was dissolved in DMSO and further diluted such that 0.1ml sample was added per plate. For each experiment an appropriate aliquot was removed from each sample vial and the remainder re-frozen and stored for further use. The Ames assay was carried out as described by Ames et al, 1975. 0.2ml S9 mix (Salamone et al, 1979) was used where appropriate. The same lot of S9 homogenate (Litton Bionetics, Maryland) was used throughout the experiments in these studies except for those (two) carried out after 011182.

Leachate Samples

Leachate samples were collected from 5 sites on or around the Upper Ottawa Street Landfill Site, namely bore holes 2 and 4, Collection Manhole, Redhill Creek upstream and downstream. In addition, a sample of Hamilton tap water was provided for comparison. The leachates were delivered to Mutatech on 3 Aug. 1982, and stored

in the refrigerator in the dark. Shortly after receipt, the pH of each sample was measured. An aliquot of each was then vacuum filtered, first through Whatman #2 filter paper then through a 0.45µ membrane filter. The final filtrates were stored as above. Before use samples were diluted .75,.5 and .25x with sterile deionized water.

Ames Assay

Top agar was concentrated such that 0.7ml could be added per plate. To this was added 2ml of sample,0.1ml cells, and, where appropriate 0.2ml S.9 mix (Salamone et al 1979). 2ml deionized water was used as a negative control. Appropriate positive controls were included in addition to (in most experiments) control plates for S 9 mix and sample sterility.

RESULTS

Air particulates

The first mutagenicity/toxicity assays either on TA98 or TA100 were carried out with the intention of using all 5 tester strains . Therefore one fifth the total volume of each sample in solution in DMSO was removed and used on one or other of these strains. It was evident, however, that the maximum doses permitted by the limited quantity of material available were insufficient to cause toxicity and a change of protocol was proposed. TA1535 and 1538 were eliminated from the study allowing the maximum dose to be increased in subsequent experiments.

Any sample then remaining was to be used in a re-test of TA98 or TA100 as appropriate at a higher concentration.

In spite of the higher doses permitted, no toxicity, as indicated by the background bacterial lawn, was observed with any extract. The most mutagenic activity was observed with TA98 and, in most cases, this was enhanced by the presence of S 9 mix (Tables II-XI). In almost all experiments some evidence of dose response was observed. Linear regression analyses were carried out on the data and a value for revertants per mg particulate matter calculated for each sample from the linear part of each dose-response curve (Tables XII-XIV). From this it was possible to calculate revertants per m^3 air sampled and these data are shown in the same table. Although artificial, these values provide a convenient way of comparing extracts. With strains TA98 and TA1537 mutagenic activity per m^3 air was greatest in the sample taken at the MoE Instrumentation Lab at Stoney Creek, although this was clearly because particulate concentration was very high at this site (Table I). In terms of mutagenicity per mg particulate, no extract exceeded the mutagenicity of the control sample -001 (Mountain Police). On re-testing, the activities of the various extracts on TA98 were not inconsistant with initial results (Tables I-XI).

Only low levels of mutagenic activity were observed with most extracts on TA100 (Tables II-XI). The only sample with which a doubling of background reversion rate was obtained was -025 (North of Site, Near Gate). (Borehole #2)

This extract was more mutagenic both in terms of revertants/mg and revertants/m^3 air than any other sample. When, however, the extract was re-tested on TA100, these levels of mutagenicity were not observed (Table XI). Extracts -002 (Blessed KateriTekakwitha School) and -004 (Bell Canada Building) were also re-tested on TA100 (Tables III and IV respectively). Results with -004 were in agreement with original observations of little or no mutagenicity. Although reduced mutagenic activity was observed with extract -002, this reduction was not so striking as that observed with -025. Reasons for the lack of reproducibility of results on re-testing of these samples on TA100 are unclear but four possible explanations are offered:

(1). The outcome of the first Ames test of sample -025 and -002 (carried out at the same time, with the same controls) was a spurious positive due to technical error.

(2) The outcome of the re-test was a false negative.

(3) A new lot of S9 homogenate was used for the re-test.

(4) The activity present in the samples declined during the 20 day period between the two experiments in spite of their being stored frozen.

The second explanation need not be considered because a third repeat (data not shown) using what remained of the sample from the final dilutions from each of the previous experiments (at 0.4ml/plate), again indicated low levels of reversion. It is not easy to dismiss any of the remaining 3 possibilities without further study.

Leachates

A doubling of background reversion rate and a clear indication of dose dependancy was shown using the sample from bore hole #4 and TA98, in both the presence and absence of S9 (Table XXI). A weak positive was also indicated with this sample and TA1538 without S9 (Table XXI). Similarly weak responses were observed in the absence of S9 with TA1538 and leachate from bore hole #2 (Table XVII) and with TA100 and the upstream sample from Redhill Creek (Table XVIII). That these increases were not the result of sample contamination was verified in each case either at the time of the experiment, by incorporation of sample into top agar, or subsequently, by shaking a 2ml aliquot with nutrient broth at 37°C.

REFERENCES

1. Ames, B.N., McCann, J., Yamasaki, E. (1975) Mutation Res. 31, 347-364.

2. Katz, M., Sakuma, T., Ho, A. (1978) Environment Sci.Tech. 12, 909-915.

3. Salamone, M.F., Heddle, J.A., Katz, M. (1979) Environment Int. 2, 37-43.

TABLE 1 COLLECTION DATA FOR AIRBORN PARTICULATES

LOCATION	Hi-vol #	Filter #	Collection Period(hrs)	Particulate Weight(mg)	Conc. Airborn Particulates ($\mu g\ m^{-3}$)
Instrumentation Laboratory (2229 Barton [illegible])	8893	-006	24	193.7	73.9
Site, East Side Borehole #4	8898	-008	24	109.5	41.6
Site, South Near Stonechurch Rd. (goose neck vents)	9495	-009	24	101.3	37.2
Bell Canada Building (on south side of Stone Church area across from dump)	9896	-004	24	97.1	36.7
Benneto School (Hanson St. N)	9739	-005	24	89.7	31.9
Site, South Side - Borehole #3	9258	-007	18	65.4	30.9
Mountain Police (west mountain between [illegible])	8834	-001	24	76.8	30.8
Site, North Side - B[illegible] 2	9246	-025	24	85.4	27.8
Firehall	9744	-010	24	68.9	26.7
Blessed Kateri Tekakwitha School	9268	-002	24	50.9	17.2

TABLE II AIR PARTICULATE ANALYSIS: REVERTANTS PER PLATE
Site: Mountain Police
High Vol #8834
Filter #29096001

Experiment #261082

Dose µg/plate	m^3	TA 98 -S9 Mean	SEM	+S9 Mean	SEM
1280	41.5	40.0	2.3	130.3	11.3
960	31.1	31.3	1.9	98.7	15.1
640	20.8	25.7	5.4	89.3	9.3
320	10.4	28.0	3.8	57.3	3.8
160	5.2	19.3	3.9	32.7	1.5
0.1ml DMSO		14.7	3.3	24.0	2.1
0.5µg 4-NQO		386.0	19.7	-	
10µg 2-AA		-		>1000	
S9 only(0.2ml)		-		0	

#271082

Dose µg/plate	m^3	TA 100 -S9 Mean	SEM	+S9 Mean	SEM
1280	41.5	146.3	6.7	149.0	5.5
960	31.1	157.3	14.8	164.3	7.3
640	20.8	147.7	2.3	174**	0.0
320	10.4	133.7	8.3	129.0	10.8
160	5.2	141.7	7.5	139.3	1.7
0.1ml DMSO		105.3	3.3	134.7	6.4
0.5µg 4-NQO		>1000		-	
10µg 2-AA		-		>1000	
S9 only(0.2ml)		-		0	

#281082

Dose µg/plate	m^3	TA 1537 -S9 Mean	SEM	+S9 Mean	SEM
1280	41.5	55.3	4.7	41.0	2.5
960	31.1	38.0	3.8	37.3	4.9
640	20.8	43.0	2.7	29.0	3.6
320	10.4	39.3	4.7	21.7	4.1
0.1ml DMSO		31.7	1.2	18.7	2.4
150µg 9AA		230.7	24.3	-	
10µg 2-AA		-		165.0	9.3
S 9 only(0.2ml)		-		0	

** only 1 plate scored

* " 2 plates "

TABLE III AIR PARTICULATE ANALYSIS: REVERTANTS PER PLATE
Site: Blessed Kateri Tekakwitha School
High Vol #9268
Filter #29096002

Experiment #181082 #131082 #201082

Dose	m^3	TA 98 -S9		+S9		Dose	m^3	TA 100 -S9		+S9		Dose	m^3	TA 1537 -S9		+S9	
µg/plate		Mean	SEM	Mean	SEM	µg/plate		Mean	SEM	Mean	SEM	µg/plate		Mean	SEM	Mean	SEM
848	49.2	32.7	7.2	93.0	5.3	509	29.5	125**		131.7	14.7	848	49.2	23.0*	9.0	21.3	5.0
636	36.9	41.0	6.5	65.0	3.8	383	22.2	108.5*	3.5	135.0	15.3	636	36.9	19.3	5.0	20.0	2.9
424	24.6	35.7	3.3	43.0	2.1	255	14.8	107.7	9.7	115.3	0.9	424	24.6	15.3	3.5	14.0	2.0
212	12.3	19.7	2.3	33.0	5.8	128	7.4	95.0	7.8	98.0	3.6	212	12.3	13.7	2.2	14.0	1.0
106	6.2	29.7	2.4	28.0	4.0	64	3.7	92.2	9.3	94.7	5.0						
0.1ml DMSO		26.7	0.6	23.3	4.3	0.1ml DMSO		82.0	6.0	80.7	4.6	0.1ml DMSO		23.0	1.0	11.3	2.3
0.5µg 4-NQO		475.3	81.0	-		0.5µg 4-NQO		484.0	35.9	-		150µg 9-AA		184.0	66.0	-	
10µg 2-AA		-		>1000		10µg 2-AA		-		>1000		10µg 2-AA		-		146.7	6.4
S9 only(0.2ml)		-		0		S9 only(0.2ml)		-		0		S9 only(0.2ml)		-		0	
						#021182											
						471	27.3	134.3	7.8	139.3	7.9						
						236	13.7	140.5	15.5	120.7	7.8						
						0.1ml DMSO		116.7	13.9	122.3	6.7						
						2µg MNNG		>1000		-							
						S9 only(0.2ml)		-		0							

TABLE IV AIR PARTICULATE ANALYSIS: REVERTANTS PER PLATE
Site : Bell Canada Building
High Vol #9892
Filter #29096004

Experiment	#081082						#081082						#161082				
		TA 98															
Dose µg/plate	m^3	-S9 Mean	SEM	+S9 Mean	SEM	Dose µg/plate	m^3	-S9 Mean	SEM	+S9 Mean	SEM	Dose µg/plate	m^3	-S9 Mean	SEM	+S9 Mean	SEM
971	26.6	26.7	2.4	27.7	2.3	971	26.6	111.3	10.2	110.0	2.1	1618	44.2	18.0	4.0	11.0	3.8
729	20.0	27.0	1.0	37.7	1.5	729	20.0	112.7	2.9	116.7	7.6	1214	33.1	15.7	1.5	12.7	1.8
486	13.3	18.3	3.8	22.0	2.3	486	13.3	119.0	13.7	113.3	4.7	809	22.1	19.0	2.5	13.0	3.2
243	6.7	16.3	3.0	21.0*	2.0	243	6.7	107.5	10.5	110.3	9.2	405	11.0	10.7	1.9	10.0	1.0
121	3.4	18.0	2.5	24.7	1.7	121	3.4	116.3	14.4	113.7	8.8	203	6.0	12.5	1.5	10.3	2.7
0.1ml DMSO		15.3	2.0	23.3	0.7	0.1ml DMSO		116.3	8.2	117.0	17.4	0.1ml DMSO		15.3	1.9	12.3	0.9
0.5µg 4-NQO		572.0	69.0	-		2.0µg MNNG		>1000		-		150µg 9-AA		59.3	25.1	-	
10µg 2-AA		-		>1000								10µg 2-AA		-		202.0	21.1
S9 only(0.2ml)		-		0								S9 only (0.2ml)		-		0	
#021182						#021182											
1438	39.2	20.3	3.7	25.7	4.4	899	24.5	135.3	7.8	125.3	4.7						
719	19.6	16.5	3.5	21.3	3.2	450	12.3	130.5	2.5	126.5	9.5						
0.1ml DMSO		13.3	2.9	22.0	2.5	0.1ml DMSO		116.7	13.9	122.3	6.7						
0.5µg 4-NQO		578.0	14.0	-		2.0µg MNNG		>1000		-							
10µg 2-AA		-		>1000		S9 only		-		0							
S9 only		-		0													

TABLE V AIR PARTICULATE ANALYSIS: REVERTANTS PER PLATE
Site: Benneto School
High Vol #9739
Filter #29096005

Experiment	#111082				
		TA 98			
Dose µg/plate	m^3	-S9 Mean	SEM	+S9 Mean	SEM
897	28.1	33.7	3.7	45.0*	12.0
673	21.1	42.3	1.3	52.7	7.6
449	14.1	31.3	1.2	48.5	1.5
224	7.0	26.0	3.5	31.0*	2.0
112	3.5	24.3	1.3	28.0	3.2
0.1ml DMSO		22.0	2.7	27.3	5.8
0.5µg 4-NQO		557.7	42.1	-	
10ug 2-AA		-		>1000	
S9 only(0.2ml)		-		0	
#021182					
1329	41.6	28.0	4.4	92.3	13.3
665	20.8	25.0*	1.0	69.0	3.6
0.1ml DMSO		13.3	2.9	22.0	2.5
0.5µg 4-NQO		578.0	14.0	-	
10µg 2-AA		-		>1000	
S9 only (0.2ml)		-		0	

#191082					
		TA 100			
Dose µg/plate	m^3	-S9 Mean	SEM	+S9 Mean	SEM
1495	46.8	147.0	11.8	158.5	12.5
1121	35.1	145.3	3.8	147.7	3.8
748	23.4	150.7	2.3	142.5	4.5
374	11.7	124.7	7.9	145.3	10.3
187	5.9	118.3	1.9	149.7	11.2
0.1ml DMSO		119.7	2.6	133.3	3.7
0.5µg 4-NQO		>1000		-	
10µg 2-AA		-		>1000	
S9 only(0.2ml)		-		0	

#201082					
		TA 1537			
Dose µg/plate	m^3	-S9 Mean	SEM	+S9 Mean	SEM
1495	46.8	16.5*	1.5	16.7	1.8
1121	35.1	22.0	1.0	25.5*	4.5
748	23.4	17.7	3.7	15.5*	0.5
374	11.7	20**	0.0	16.7	2.9
0.1ml DMSO		23.0	1.0	11.3	2.3
150µg 9-AA		184.0	66.0	-	
10µg 2-AA		-		146.7	6.4
S9 only(0.2ml)		-		0	

TABLE VI AIR PARTICULATE ANALYSIS: REVERTANTS PER PLATE
Site: Ministry of Environment Instrument Shop, Stoney Creek
High Vol #8895
Filter #29096006

Experiment		#031082			
		TA 98			
		-S9		+S9	
Dose µg/plate	m^3	Mean	SEM	Mean	SEM
1937	26.2	86.0	7.8	170.0	6.7
1453	19.6	74.7	6.8	124.7	10.0
968	13.1	67.7	5.0	116.3	10.0
484	6.5	43.3	4.7	63.7	3.7
193	2.6	29.5	8.5	33.5	5.5
58	0.8	18.0	2.5	26.3	4.9
0.1ml DMSO		16.0	3.0	22.0	0.0
0.5µg 4-NQO		492.0	12.0	-	
7.0µg 2-AA		-		>1000	
S9 only (0.2ml)		-		0	
#021182					
2869	38.8	89.0	8.9	448.7	39.7
1434	19.4	51.0	1.0	286.7	13.2
0.1ml DMSO		13.3	2.9	22.0	2.5
0.5µg 4-NQO		578.0	14.0	-	
7µg 2-AA		-		>1000	
S9 only (0.2ml)		-		0	

		#061082			
		TA 100			
		-S9		+S9	
Dose µg/plate	m^3	Mean	SEM	Mean	SEM
1937	26.2	125.3	6.4	173.7	9.9
1453	19.6	106.3	4.3	137.3	2.4
968	13.1	113.3	9.6	147.7	5.8
484	6.5	142.0	1.0	132.0	8.7
242	3.3	111.0	6.6	108.7	8.6
0.1ml DMSO		95.3	8.9	105.0	7.8
1.2µg MNNG		>1000		-	
10µg 10-AA		-		>1000	
S9 only (0.2ml)		-		0	

		#161082			
		TA 1537			
		-S9		+S9	
Dose µg/plate	m^3	Mean	SEM	Mean	SEM
3228	43.7	41.7	3.8	62.0	9.8
2421	32.7	34.0	3.1	52.3	0.7
1614	21.8	30.0	4.6	47.0	2.6
807	10.9	18.7	3.5	29.3	1.9
404	5.5	17.3	3.3	15.5	0.5
0.1ml DMSO		15.3	1.9	12.3	0.9
150µg 9-AA		59.3	25.1	-	
10µg 10-AA		-		202.0	21.1
S9 only (0.2ml)		-		0	

TABLE VII AIR PARTICULATE ANALYSIS: REVERTANTS PER PLATE
Site: South Side Landfill
High Vol #9258
Filter #29096007

Experiment #091082

Dose µg/plate	m³	TA 98 -S9 Mean	 SEM	 +S9 Mean	 SEM
654	21.2	27.0	7.0	31.7	6.4
491	15.9	21.0	1.5	27.0	3.0
327	10.6	17.0*	0.0	22.7	2.2
164	5.3	13.3	2.2	18.7	0.9
82	2.6	13.0	2.7	22.0	3.6
0.1ml DMSO		13.7	2.3	19.3	3.0
0.5µg 4-NQO		595.3	20.3	-	
10µg 2-AA		-		>1000	
S9 only(0.2ml)		-		0	
#021182					
848	27.5	19.5*	0.5	52.0*	2.0
424	13.7	19.0*	3.0	41.3	4.7
0.1ml DMSO		13.3	2.9	22.0	2.5
0.5µg 4-NQO		578.0	14.0	-	
10ug 2-AA		-		>1000	
S9 only (0.2ml)		-		0	

Experiment #151082/191082

Dose µg/plate	m³	TA 100 -S9 Mean	 SEM	 +S9 Mean	 SEM
1090	35.3	112.7	9.2	144.0	17.1
818	26.5	109.3	6.6	53.0(a)	1.2
545	17.6	108.3	4.4	136.3	14.4
273	8.8	126.0	5.0	145.3	11.7
136	4.4	115.7	8.8	151.0	5.5
0.1ml DMSO		114.3	0.9	133.3	3.7
2µg MNNG		>1000		-	
10µg 2-AA		-		>1000	
S9 only (0.2ml)		-		0	

Experiment #201082

Dose µg/plate	m³	TA 1537 -S9 Mean	 SEM	 +S9 Mean	 SEM
1090	35.3	-		18.0	0.5
818	26.5	-		21.0	1.2
545	17.6	-		19.0	3.1
273	8.8	-		14.0	2.1
0.2ml DMSO		-		11.3	2.3
150µg 9-AA		184.0	66.0	-	
10µg 2-AA		-		146.7	6.4
S9 only(0.2ml)		-		0	

(a) Toxicity Observed

TABLE VIII AIR PARTICULATE ANALYSIS: REVERTANTS PER PLATE
Site: E.Side Landfill
High Vol #8893
Filter #29096008

Experiment #261082

Dose µg/plate	m^3	TA 98 -S9 Mean	SEM	+S9 Mean	SEM
1825	43.9	48.3	6.01	152.7	9.7
1369	32.9	36.0	4.0	75.3	8.1
913	22	30.7	0.7	67.3	3.7
456	11	26.7	2.8	36.3	3.3
228	5.5	17.3	1.9	30.3	4.1
0.1ml DMSO		14.7	3.3	24.0	2.1
0.5µg 4-NQO		386.0	19.7	-	
10µg 2-AA		-		>1000	
S9 only(0.2ml)		-		0	

#271082

Dose µg/plate	m^3	TA 100 -S9 Mean	SEM	+S9 Mean	SEM
1825	43.9	123.0	2.5	156.0	8.5
1369	32.9	140.3	7.4	161.7	16.7
913	22	147.3	9.3	139.3	10.5
456	11	153.7	10.2	146.3	13.0
228	5.5	143.3	2.4	140.3	6.6
0.1ml DMSO		105.3	3.3	134.7	6.4
0.5µg 4-NQO		>1000		-	
10µg 2-AA		-		>1000	
S9 only(0.2ml)		-		0	

#281082

Dose µg/plate	m^3	TA 1537 -S9 Mean	SEM	+S9 Mean	SEM
1825	43.9	53.7	3.5	39.7	2.9
1369	32.9	40.0	2.7	31.3	1.7
913	22	34.0	5.0	22.0	4.9
456	11	18.0	2.1	19.7	4.3
0.1ml DMSO		31.7	1.2	18.7	2.4
150µg 9-AA		230.7	24.3	-	
10µg 2-AA		-		165.0	9.3
S9 only (0.2ml)		-		0	

TABLE IX AIR PARTICULATE ANALYSIS: REVERTANTS PER PLATE
Site: South East Side Landfill
High Vol #9495
Filter #29096009

Experiment	#261082					#271082						#281082					
		TA 98						TA 100						TA 1537			
Dose µg/plate	m^3	-S9 Mean	SEM	+S9 Mean	SEM	Dose µg/plate	m^3	-S9 Mean	SEM	+S9 Mean	SEM	Dose µg/plate	m^3	-S9 Mean	SEM	+S9 Mean	SEM
1688	45.4	50.7	9.7	153.7	12.8	1688	45.4	167.0	8.7	169.0	6.8	1688	45.4	35.3	2.7	44.3	5.8
1266	34.0	36.3	5.4	120.0	6.7	1266	34.0	150.3	8.7	174.3	8.9	1266	34.0	33.3	8.3	40.0	6.5
844	22.7	38.7	2.4	78.7	13.0	844	22.7	137.7	9.7	160.0	4.6	844	22.7	38.3	3.9	32.7	3.4
422	11.3	25.0	0.6	-		422	11.3	134.3	4.4	131.0	4.6	422	11.3	31.7	1.7	29.3	1.5
						211	5.7	138.3	4.5	118.0	6.1	0.1ml DMSO		31.7	1.2	18.7	2.4
0.1ml DMSO		14.7	3.3	24.0	2.1	0.2ml DMSO		105.3	3.3	134.7	6.4	150µg 9-AA		230.7	24.3	-	
0.5µg 4-NQO		386.0	19.7	-		0.5µg 4-NQO		>1000		-		10µg 2-AA		-		165.0	9.3
10µg 2-AA		-		>1000		10µg 2-AA		-		>1000		S9 only(0.2ml)		-		0	
S9 only(0.2ml)		-		0		S9 only (0.2ml)		0		-							

TABLE X AIR PARTICULATE ANALYSIS: REVERTANTS PER PLATE

Site: Firehall
High Vol #9744
Filter #29096010

Experiment	#111082					#191082						#211082					
		TA 98						TA 100						TA 1537			
Dose µg/plate	m^3	-S9 Mean	SEM	+S9 Mean	SEM	Dose µg/plate	m^3	-S9 Mean	SEM	+S9 Mean	SEM	Dose µg/plate	m^3	-S9 Mean	SEM	+S9 Mean	SEM
689	25.8	38.0	3.0	58.0	0.6	1148	42.9	138.7	8.1	141.3	13.1	1148	42.9	23.7	3.8	26.7	1.3
517	19.3	32.7	2.9	36.0	1.5	861	32.2	115.3	14.8	149.7	2.6	861	32.2	28.7	1.8	26.3	4.3
345	12.9	33.0	1.5	36.3	1.2	574	21.5	133.7	8.6	151.7	9.7	574	21.5	14.0	2.5	19.0	2.5
172	6.5	21.7	2.4	23.3	1.3	287	10.7	145.3	3.2	136.0	3.6	287	10.7	25.3	1.2	19.0	3.6
86	3.2	19.3	1.8	26.7	1.9	144	5.4	117.0	10.5	140.0	15.7						
0.1ml DMSO		22.0	2.7	27.3	5.8	0.1ml DMSO		119.7	2.6	133.3	3.7	0.1ml DMSO		15.3	0.88	17.3	1.9
0.5ug 4-NQO		557.7	42.0	-		0.5ug 4-NQO		>1000		-		150µg 9-AA		228.7	25.3	-	
10ug 2-AA		-		>1000		10ug 2-AA		-		>1000		10µg 2-AA		-		181.0	16.6
S9 only (0.2ml)		-		0		S9 only(0.2ml)		-		0		S9 only(0.2ml)		-		1	
#021182																	
1020	38.2	25.0	2.0	69.3	7.1												
510	19.1	24.0	1.0	47.3	4.3												
0.1ml DMSO		13.3	2.9	22.0	2.5												
0.5µg 4-NQO		578.0	14.0	-													
10µg 2-AA		-		>1000													
S9 only(0.2ml)		-		0													

TABLE XI AIR PARTICULATE ANALYSIS: REVERTANTS PER PLATE

Site: North Gate, Landfill
High Vol #9246
Filter #29096025

Experiment #181082

TA 98 Dose µg/plate	m^3	-S9 Mean	SEM	+S9 Mean	SEM
1423	51.1	47.0	3.8	71.3	4.5
1068	38.4	37.0	2.1	60.0	5.9
712	25.6	37.7	2.2	52.0	5.5
356	12.8	31.7	2.2	49.0	5.2
178	6.4	25.0	0.6	31.3	2.6
0.1ml DMSO		26.7	0.6	23.3	4.3
0.5µg 4-NQO		475.3	81.0	-	
10µg 2-AA		-		>1000	
S9 only(0.2ml)		-		0	

Experiment #131082

TA 100 Dose µg/plate	m^3	-S9 Mean	SEM	+S9 Mean	SEM
854	30.7	118.5*	5.5	272.5*	0.5
641	23.0	113.0*	11.0	248.0*	5.0
427	15.3	110.0	3.8	148.3	6.8
214	7.7	97.0	3.8	99.7	4.3
107	3.8	91.7*	10.1	109.0	6.0
0.1ml DMSO		82.0	6.0	80.7	4.5
0.5µg 4-NQO		484.0	35.9	-	
10µg 2-AA		-		>1000	
S9 only (0.2ml)		-		0	
#021182					
949	34.1	125.3	8.8	125.9	13.9
475	17.0	132.5*	3.5	130.3	5.8
0.1ml DMSO		116.7	13.9	122.3	6.7
2µg MNNG		>1000		-	
S9 only (0.2ml)		-		0	

Experiment #211082

TA 1537 Dose µg/plate	m^3	-S9 Mean	SEM	+S9 Mean	SEM
1423	51.1	23.3	2.2	24.0	3.5
1068	38.4	24.3	3.5	35.3	2.7
712	25.6	21.7	1.2	25.3	2.6
356	12.8	18.3	2.4	16.0	1.7
0.1ml DMSO		15.3	0.9	17.3	1.9
150µg 9-AA		228.7	25.3	-	
10µg 2-AA		-		181.0	16.6
S9 only(0.2ml)		-		0	

TABLE XII MUTAGENICITY VALUES FROM REGRESSION ANALYSIS FOR TA 98

Location	Revertants/mg		Revertants/m^3		Correlation Coefficent	
	-S9	+S9	-S9	+S9	-S9	+S9
Mountain Police (control)	17	82	.5	2.5	.94	.99
Blessed Kateri Tekakwitha School	25	80	.4	1.4	.77	.97
Bell Canada Building	14	16	.5	.6	.86	.70
Benneto School (control)	29	43	.9	1.4	.97	.96
MoE Instrumentation Lab(control)	37	76	2.7	5.7	.98	.99
Site,S.Side	21	18	.7	.6	.96	.93
Site,E.Side	17	64	.7	2.7	.98	.94
Site,S.E.Side(Stonechurch Rd.)	20	77	.7	2.9	.96	1.0
Firehall	27	42	.7	1.1	.94	.88
Site, N.Side	14	31	.4	.9	.95	.96

TABLE XIII MUTAGENICITY VALUES FROM REGRESSION ANALYSIS FOR TA 100

Location	Revertants/mg		Revertants/m^3		Correlation Coefficient	
	-S9	+S9	-S9	+S9	-S9	+S9
Mountain Police (control)	43	41	1.3	1.3	.84	.79
Blessed KateriTekakwitha School	75	135	1.3	2.3	.97	.99
Bell Canada Building	-	-	-	-	-.28	-.34
Benneto School (control)	22	11	1.0	0.3	.86	.74
MoE Instrumentaion Lab.(control)	-	31	-	2.3	.25	.92
Site S.Side	-	-	-	-	-.44	.1
Site E.Side	-	16	-	1.0	.07	.81
Site S.E.Side (Stonechurch Rd.)	28	41	1.0	1.5	.89	.9
Firehall	-	21	-	1.0	.26	.9
Site N.Side	41	240	1.2	6.7	.96	.97

TABLE XIV MUTAGENICITY VALUES FROM REGRESSION ANALYSIS FOR TA 1537

Location	Revertants/mg		Revertants/m^3		Correlation Coefficient	
	-S9	+S9	-S9	+S9	-S9	+S9
Mountain Police (control)	7	19	.2	.6	.62	.99
Blessed KateriTekakwitha School	0	12	-	.2	.21	.96
Bell Canada Building	0	0	0	0	.59	.2
Benneto School (control)	0	11	0	.4	-.29	.89
MoE Instrumentation Lab (control)	9	16	.6	1.2	.99	.98
Site, S.Side	ND	12	ND	.4	ND	.99
Site, E.Side	15	12	.4	.3	.8	.94
Site, S.E. Side Stonechurch Rd.	0	15	0	.6	.5	.98
Firehall	10	9	.3	.2	.51	.92
Site N.Side	9	18	.2	.5	.99	.92

TABLE XV CHARACTERISTICS OF LEACHATE SAMPLES

Site	pH	Characteristics
Upstream, Red Hill Creek	8.0	Clear, some sediment
Landfill, Borehole #2	8.4	Dark brown, oily, black sediment
Downstream, Red Hill Creek	8.3	Clear, some sediment
Hamilton Tap Water	7.8	Clear
Landfill, Collection Manhole	8.3	Dark brown, particles in suspension, green tinge.
Landfill, Borehole #4	8.2	Black, heavy sediment oily.

TABLE XVI LEACHATE ANALYSIS: REVERTANTS PER PLATE

Site Upstream Red Hill Creek

Experiment#	050882				100982				080982				170882				230982			
	TA 98				TA 100				TA 1535				TA 1537				TA 1538			
Dose	-S9		+S9		-S9		+S9		-S9		+S9		-S9		+S9		-S9		+S9	
ml/plate	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
2.0	21.3	1.8	31.0	1.5	113.0	11.6	107.3	8.0	31.3	3.2	16.3	2.4	14.0	2.5	10.7	2.4	2.0	0.0	2.7	1.3
1.5	17.7	0.3	34.0	1.2	106.3	6.3	100.0	4.5	27.3	5.8	16.3	2.6	9.7	0.3	11.7	1.5	1.3	0.9	2.0	1.0
1.0	19.7	2.0	38.3	3.3	119.3	7.7	130.7	12.1	33.3	5.0	13.3	2.6	19.7	7.5	17.7	2.4	3.7	0.7	2.0	0.6
0.5	21.7	4.7	31.3	4.5	133.3	12.0	125.7	13.0	37.0	3.2	11.3	2.0	13.3	2.9	11.7	1.2	2.0	1.2	2.3	1.5
0.25	20.7	0.3	28.7	3.4	-		-		-		-		-		-		-		-	
2ml deionized H_2O	20.8	1.3	29.5*	3.5	118.3	14.1	117.5*	4.5	32.3	1.3	14.7	0.3	18.3	3.8	12.7	3.3	5.0	1.7	2.7	1.5
0.5µg 4-NQO	596.0*	204.0*	-		-		-		-		-		-		-		-		-	
150µg 9-AA	-		-		-		-		-		-		-		-		-		-	
2µg MNNG	-		-		>1000		-		>1000		-		-		-		-		-	
10µg 2-AA	-		>1000		-		>1000		-		214.3	18.8	-		173.0	33.0	-		217.0	3.0
S9 only	-		-		-		0		-		0		-		0		-		0	
2ml Sample only	-		-		0		-		0		-		0		-		-		-	

TABLE XVII LEACHATE ANALYSIS: REVERTANTS PER PLATE

Site Borehole #2

Experiment	060882				220982				090982				300982				091182			
	TA 98				TA 100				TA 1535				TA 1537				TA 1538			
	-S9		+S9		-S9		+S9		-S9		+S9		-S9		+S9		-S9		+S9	
Dose ml/plate	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
2.0	24.7	1.2	23.3	2.9	112.7	5.6	142.7	12.6	39.7	4.7	24.0	2.7	8.5*	2.5	-ND-		4.0	1.0	3.7	1.9
1.5	19.7	3.5	18.3	1.5	101.7	12.7	124.3	3.7	41.0	2.5	24.0	1.2	19.0	5.6	18.5	2.5	3.7	1.5	3.0	1.5
1.0	19.3	2.9	28.3	2.9	101.3	9.4	118.7	3.8	42.3	0.7	22.0	1.2	16.0	5.1	13.3	1.2	1.0	0.6	1.7	0.3
0.5	23.7	1.8	29.0	2.1	99.0	8.3	125.3	8.5	43.3	4.3	16.7	4.2	27.7	4.1	15.0	2.0	1.7	0.3	2.0	0.6
0.25	23.0	2.9	33.0	1.2	-		-		-		-		-		-		-		-	
2ml deionized H_2O	21.7	1.2	29.0	2.3	108.0	4.2	114.8	8.1	49.0	6.7	15.3	0.3	21.3	4.1	15.8	2.9	2.0	1.0	3.0	1.0
0.5µg 4-NQO	560.0	56.8	-		-		-		-		-		-		-		55.9	7.9	-	
2µg MNNG	-		-		>1000		-		>1000		-		-		-		-		-	
150µg 9-AA	-		-		-		-		-		-		105.0*	27.0	-		-		-	
10ug 2-AA	-		>1000		-		>1000		-		217.7	18.2	-		284.0	30.3	-		TOX	
S9 only	-		-		-		0		-		ND		-		0		-		0	
Sample only	-		-		0		-		0		-		0		-		0		-	

TABLE XVIII LEACHATE ANALYSIS: REVERTANTS PER PLATE

Site Downstream, Red Hill Creek

Experiment	050882				100982				080982				170882				230982			
	TA 98				TA 100				TA 1535				TA 1537				TA 1538			
Dose ml/plate	-S9 Mean	SEM	+S9 Mean	SEM	-S9 Mean	SEM	+S9 Mean	SEM	-S9 Mean	SEM	+S9 Mean	SEM	-S9 Mean	SEM	+S9 Mean	SEM	-S9 Mean	SEM	+S9 Mean	SEM
2.0	22.3	1.5	34.3	2.9	137.0	4.2	155.0	0.6	34.3	4.8	15.0	1.7	12.0	2.7	10.3	3.2	4.3	0.9	4.0	1.5
1.5	24.0	0.0	27.7	1.7	154.0	10.1	138.3	8.4	31.3	2.3	15.0	1.0	7.3	0.9	11.0	1.2	3.3	0.7	3.0	1.0
1.0	22.0	1.5	32.0	6.0	164.0	10.8	128.0	2.7	35.5*	3.5	17.3	1.2	8.7	2.7	19.0	3.5	5.7	0.7	1.3	1.3
0.5	24.0	1.0	36.0	3.0	168.0	34.0	132.3	8.3	31.0	2.9	16.3	3.9	8.0	2.1	18.0	1.5	3.3	0.3	3.0	0.6
0.25	25.7	3.5	28.3	0.9	-		-		-		-		-		-		-		-	
2ml deionized H_2O	20.8	1.3	29.5*	3.5	118.3	14.0	117.5*	4.5	32.3	1.3	14.7	0.3	18.3	3.8	12.7	3.3	5.0	1.7	2.7	1.5
0.5µg 4-NQO	596.0	204.0	-		-		-		-		-		-		-		-		-	
2ug MNNG	-		-		>1000		..		>1000		-		-		-		-		-	
150ug 9AA	-		-		-		-		-		-		-		-		-		-	
10ug 2-AA	-		>1000		-		>1000		-		214.3	18.8	-		173.0*	33.0	-		217.0*	6.3
Sample only	-		-		0		-		0		-		0		-		-		-	
S9 only	-		-		-		0		-		0		-		0		-		0	

TABLE XIX LEACHATE ANALYSIS: REVERTANTS PER PLATE

Site Hamilton Tap Water

Experiment#	050882				100982				080982				300982				230982			
	TA 98				TA 100				TA 1535				TA 1537				TA 1538			
Dose ml/plate	-S9 Mean	SEM	+S9 Mean	SEM	-S9 Mean	SEM	+S9 Mean	SEM	-S9 Mean	SEM	+S9 Mean	SEM	-S9 Mean	SEM	+S9 Mean	SEM	-S9 Mean	SEM	+S9 Mean	SEM
2.0	23.7	2.6	33.0	3.5	133.7	6.5	115.3	13.4	43.0	2.1	17.3	3.5	15.3	1.7	21.0	2.5	3.3	1.2	3.7	0.9
1.5	19.0	3.5	33.3	0.9	124.0	6.2	107.3	7.6	22.0	3.8	18.0	1.2	19.0	4.5	20.5*	5.5	2.0	0.6	2.0	0.6
1.0	18.0	1.0	20.7	2.2	133.3	6.9	137.7	14.1	25.3	1.5	29.7	7.2	17.7	5.0	21.0	5.5	2.7	1.2	1.3	0.3
0.5	16.0	2.1	26.7	3.5	142.7	9.9	136.5*	2.5	33.3	0.7	16.7	3.2	23.0	4.2	15.7	2.7	2.5	0.5	1.3	0.3
0.25	20.7	6.8	-		-		-		-		-		-		-		-		-	
2ml deionized H_2O	20.8	1.3	29.5*	3.5	118.3	14.1	117.5	14.5	32.3	1.3	14.7	0.3	21.3	4.1	15.8	2.9	5.0	1.7	2.7	1.5
0.5µg 4-NQO	596.0*	204.0*	-		-		-		-		-		-		-		-		-	
2µg MNNG	-		-		>1000		-		>1000		-		-		-		-			
150 µg 9-AA	-		-		-		-		-		-		105.0*	27.0	-		-		-	
10µg 2-AA	-		>1000		-		>1000		-		214.3	18.8	-		284.0	30.3	-		217.0*	3.0
Sample only	-		-		0		-		0		-		0		-		-			
S9 only	-		-		-		-		-		0		-		0		-		0	

TABLE XX LEACHATE ANALYSIS: REVERTANTS PER PLATE

Site Collection Manhole

Experiment # 060822 220982 090982 300982 230982

Dose ml/plate	TA 98 -S9 Mean	SEM	+S9 Mean	SEM	TA 100 -S9 Mean	SEM	+S9 Mean	SEM	TA 1535 -S9 Mean	SEM	+S9 Mean	SEM	TA 1537 -S9 Mean	SEM	+S9 Mean	SEM	TA 1538 -S9 Mean	SEM	+S9 Mean	SEM
2.0	21.7	5.2	27.0	3.0	97.7	6.8	116.7	7.8	45.7	3.5	24.3	5.6	26.0*	1.0	13.5*	3.5	3.3	0.9	4.0	1.2
1.5	19.0	1.2	27.7	0.9	88.3	4.4	110.7	6.0	43.0	2.5	20.0	2.1	18.5*	2.5	14.5	1.5	2.3	0.7	3.5*	2.5
1.0	24.7	3.8	34.0	3.1	90.3	2.9	112.7	4.5	50.3	8.4	26.5*	0.5	22.5*	4.5	17.0*	0.0	2.7	1.7	2.3	0.3
0.5	32.0	2.0	30.7	1.3	101.0	4.2	118.7	2.0	47.3	4.8	18.0	1.5	19.3	4.8	TOX		2.0	0.6	3.5	0.5
0.25	20.0	1.0	36.3	1.7	-		-		-		-		-		-		-		-	
2ml deionized H_2O	21.7	1.2	29.0	2.3	108.0	4.2	114.8	8.1	49.0	6.7	15.3	0.3	21.3	4.1	15.8	2.9	5.0	1.7	2.7	1.5
0.5μg 4-NQO	560.0	56.8	-		-		-		-		-		-		-		-		-	
2μg MNNG	-		-		>1000		-		>1000		-		-		-		-		-	
150μg 9-AA	-		-		-		-		-		-		105.0*	27.0	-		-		-	
10μg 2-AA	-		>1000		-		>1000		-		217.7	18.2	-		284.0	30.3	-		217.0	3.0
Sample only	-		-		0		-		0		-		0		-		-		-	
S9 only	-		-		-		0		-		-		-		0		-		0	

TABLE XXI LEACHATE ANALYSIS: REVERTANTS PER PLATE

Site Borehole #4

Experiment#	060882				220982				090982				300982				230982			
	TA 98				TA 100				TA 1535				TA 1537				TA 1538			
Dose ml/plate	-S9 Mean	SEM	+S9 Mean	SEM	-S9 Mean	SEM	+S9 Mean	SEM	-S9 Mean	SEM	+S9 Mean	SEM	-S9 Mean	SEM	+S9 Mean	SEM	-S9 Mean	SEM	+S9 Mean	SEM
2.0	41.0	4.4	39.3	3.3	113.7	2.6	114.7	1.7	44.0	9.5	22.0	1.5	24.0	3.0	21.7	3.3	5.3	1.7	7.3	1.7
1.5	51.3	1.9	51.3	4.1	101.0	4.5	129.7	10.7	34.0	2.1	25.0	1.5	23.0	2.5	25.0	7.1	4.0	0.0	6.3	2.4
1.0	51.3	1.2	65.3	8.7	95.0	0.6	113.3	6.5	34.0	6.4	22.3	2.8	24.0	4.0	26.7	2.3	2.7	1.2	2.0*	1.0
0.5	43.0	4.0	58.0	4.0	115.7	5.6	96.0	4.5	52.0*	6.0	22.7	1.5	33.5*	1.5	21.0	2.7	1.7	0.3	2.3	0.9
0.25	33.7	2.4	52.7	0.9	-		-		-		-		-		-		-		-	
2ml deionized H_2O	21.7	1.2	29.0	2.3	108.0	4.2	114.8	8.1	49.0	6.7	15.3	0.3	21.3	4.1	15.7	2.9	5.0	1.7	2.7	1.5
0.5ug 4-NQO	560.0	56.8	-		-		-		-		-		-		-		-		-	
2ug MNNG	-		-		>1000		-		>1000		-		-		-		-		-	
150ug 9-AA	-		-		-		-		-		-		105.0*	27.0	-		-			
10ug 2-AA	-		>1000		-		>1000		-		217.7	18.2	-		284.0	30.3	-		217.0	3.0
Sample only	-		-		0		-		0		-		0		-		-		-	
S9 only	-		-		-		0		-		-		-		0		-		0	

STRAIN CHECK

DATE 8th Oct 82.
STOCK Set up 7 Oct from stocks frozen 7 Oct.

STRAIN	TA98	TA100	TA1535	TA1537	TA1538
GROWTH IN PRESENCE OF HIS	✓	✓	✓	✓	✓
GROWTH IN ABSENCE OF HIS	✗	✗	✗	✗	✗
CRYSTAL VIOLET: ZONE OF INHIBITION	✓	✓	✓	✓	✓
UV,6SEC GOWTH ON ONLY HALF PLATE			✓	✓	✓
UV,8SEC	✓	✓			
AMPICILLIN INHIBITION	✗	✗	✓	✓	✓
SPONT REVERSION:	12	110	19 29	4 0	12 10

SPOT TEST

INDUCED REVERSION:

	TA98	TA100	TA1535	TA1537	TA1538
MNNG	✓	✓	✓	✓	✓
4NQO	✓	✓	✓	✗	✓
9AA	✗	✓	✓	✗	✗

11 Oct 82.

16/10 1537 vs 150µg/plate 9AA - clear +ve

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